A PROCESS FOR THE PRODUCTION OF EGG YOLK ANTIBODIES FOR ORGANOCHLORINE PESTICIDES

FIELD OF INVENTION

The present invention relates to the production of egg yolk antibodies binding to small molecule organochlorine pesticides. The present invention also relates to periodic immunization of the poultry birds with a desired hapten-protein conjugate in the breast muscle.

BACKGROUND OF THE INVENTION

The organochlorine pesticides are large class of insecticides that generally accumulate in the tissues and are difficult to degrade as they have very long half-life periods.

Organochlorine insecticides are used widely for treatment of crops such as cereals, fruits, vegetables, legumes, cotton, coffee and tea, forest trees, sanitation and domestic mosquito/ malaria control programs.

Small organic molecules such as pesticides are not by themselves immunogenic, but can be made immunogenic following chemical conjugation to a suitable carrier such as a protein. The site and nature of conjugation and carrier protein can influence the specificity and affinity of antibodies produced, in a manner not fully able to be predicted by those skilled in the art. For example, preparation of DDA - GABA (a compound closely related to the hapten used in the present invention coupled with-bovine serum albumin) did not yield conjugates producing antisera with useful reaction with – DDT. (Amitarani, B.E., Akmal Pasha and Karanth N.G.K. (1998, unpublished data).

In recent years, techniques of producing antibodies (polyclonal and monoclonal) have been developed which make it possible to obtain homogeneous, highly specific antibodies. Generally, polyclonal antibodies are used extensively in diagnostics industry. Most

commonly they are raised in mammals such as rabbits, mice, rats, horses and goats. This form of antibody production has several disadvantages – large mammals are expensive to maintain, while small mammals yield small quantities of antibody. In addition there is a requirement for periodic drawing of blood from the animals (Deignan et al. 2000)). The amount of IgG (antibody) obtained is usually between 3-8 mg/ ml of serum. The method also involves bleeding of the rabbit several times to obtain the antibodies, as the titer is highest only between the 8^{th} - 10^{th} day after 2 – 3 boosters. Monoclonal antibodies are produced by immunizing an animal with a protein, obtaining antibody-producing cells from the animal, and fusing the antibody-producing cells with strains of myeloma cells, i.e., tumor cells, to produce hybridomas, which are isolated and cultured as monoclones. The monoclonal hybridomas may either be cultured in vitro or from the cells, ascitic fluid, or serum of a tumor-bearing host animal. Since each antibody-producing cell generates a single unique antibody, each monoclonal culture of hybridomas produces a homogeneous antibody. Not all of the hybridoma clones, which result from fusing myeloma cells with antibody-producing cells, are specific for the desired pesticide (or for functional groups upon the pesticide characteristic of that class of molecules), since many of the hybridomas will make antibodies, which the inoculated animal has produced to react with other foreign substances. Even antibodies against the subject antigen will differ from clone to clone, since antibodies produced by different cells may react with different antigenic determinants of the same molecule. From each clone, therefore, it is necessary to obtain the resulting antibody and test its reactivity with the subject pesticides and to test its specificity by determining which particular organochlorine pesticide it recognizes. Further, only certain antibodies or antisera function in specific immunoassay formats or configurations.

United States Patent Nos. 4,387,272 and 4,550,019 to Polson; and Losch, U claimed production of hen egg yolk antibodies and has been used in a number of applications for passive transfer of immunity.

United States Patent No. 4,748,018 to Stolle, et al. discloses a method of passive immunization against bacterial infection comprising a preliminary development of

tolerance to HEY by repeated oral ingestion of egg yolk, followed by parenteral injection of HEY antibody to a selected bacterial antigen.

United States Patent No. 5,080,895 to Tokoro discloses prevention of E. Coli diarrhoea in newborn piglets by oral administration of anti-bacterial hen egg yolk antibodies.

Reference is made to Hamada, S., Infection and Immunity 59(11): 4161-4167 (1991); and Otake, S., J. Dental Research 70(3): 162-166 (1991) reproduced the results of Beck in protecting rats against dental caries by means of passive immunization with orally administered hen egg yolk antibodies against S. mutans.

Reference is made to Bartz, C. et al., J. Infectious Disease 142(3): 439-441 (1980) prevented murine rotaviral infection in mice by the oral administration of the water-soluble fraction of the eggs of immunized hens

Reference is made to Yokoyama, H., et al. Infection and Immunity 60(3): 998-1007 (1992) succeeded in passively protecting neonatal piglets from fatal enterotoxigenic E. coli infection by oral administration of a crude yolk immunoglobulin fraction from the eggs of immunized hens.

Reference is made to the animal studies of Yolken, R. et al., Pediatrics 81(2): 291-295 (1988); and Journal Clint. Immunol. 10(6): 80S-87S (1990), proposed the oral administration of antiviral HEY immunoglobulin for the prevention and treatment of enteric infections, including rotaviral infection in humans. Methods and formulations for the oral administration of immune globulin are known (U.S. Pat. No. 4,477,432 to Hardie). However, there are no reports on the production of egg yolk antibodies for a small molecule such as a pesticide.